(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 February 2002 (28.02.2002)

PCT

(10) International Publication Number
WO 02/16658 A1

(51) International Patent Classification[7]: C12Q 3/00, 1/68, C12P 21/04, C12N 15/00, 5/00, C07H 21/04

(21) International Application Number: PCT/US01/26039

(22) International Filing Date: 17 August 2001 (17.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/227,681 24 August 2000 (24.08.2000) US
60/227,682 24 August 2000 (24.08.2000) US

(71) Applicant *(for all designated States except US)*: **SIERRA SCIENCES, INC.** [US/US]; Suite 130, 250 South Rock Boulevard, Reno, NV 89502 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ANDREWS, William, H.** [US/US]; 3430 Rosalinda Drive, Reno, NV 89503 (US). **FOSTER, Christopher, A.** [US/US]; 2806 Panay Court, Carmichael, CA 95608 (US). **FRASER, Stephanie** [US/US]; 1011 Tyler Way, Sparks, NV 89431 (US). **MOHAMMADPOUR, Hamid** [IR/US]; 4748 Cedar Hill Lane, Reno, NV 89509 (US).

(74) Agent: **FIELD, Bret, E.**; Bozicevic, Field & Francis, LLP, Suite 200, 200 Middlefield Road, Menlo Park, CA 94025 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/16658 A1

(54) Title: METHODS AND COMPOSITIONS FOR MODULATING TELOMERASE REVERSE TRANSCRIPTASE (TERT) EXPRESSION

(57) Abstract: Methods and compositions are provided for modulating, and generally upregulating, the expression of telomerase reverse transcriptase (TERT) by modulating Myc Repeat region repression of TERT expression. The subject methods and compositions find use in a variety of different applications, including the immortalization of cells, the production of reagents for use in life science research, therapeutic applications; therapeutic agent screening aplications; and the like. In further describing the subject invention, the methods and compositions of the invention are described first in greater detail, followed by a review of the various applications in which the subject invention finds use.

METHODS AND COMPOSITIONS FOR MODULATING TELOMERASE REVERSE TRANSCRIPTASE (TERT) EXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

Pursuant to 35 U.S.C. § 119 (e), this application claims priority to the filing dates of the United States Provisional Patent Application Serial Nos. 60/227,682 and 60/227,681, both filed August 24, 2000; the disclosures of which are herein incorporated by reference.

INTRODUCTION

Field of the Invention

The field of this invention is the telomerase reverse transcriptase gene, specifically the regulation of the expression thereof.

Background of the Invention

Telomeres, which define the ends of chromosomes, consist of short, tandemly repeated DNA sequences loosely conserved in eukaryotes. Human telomeres consist of many kilobases of (TTAGGG)n together with various associated proteins. Small amounts of these terminal sequences or telomeric DNA are lost from the tips of the chromosomes during S phase because of incomplete DNA replication. Many human cells progressively lose terminal sequence with cell division, a loss that correlates with the apparent absence of telomerase in these cells. The resulting telomeric shortening has been demonstrated to limit cellular lifespan.

Telomerase is a ribonucleoprotein that synthesizes telomeric DNA. Human telomerase is made up of two components: (1) an essential structural RNA (TER) (where the human component is referred to in the art as hTER); and (2) a catalytic protein (telomerase reverse transcriptase or TERT) (where

the human component is referred to in the art as hTERT). Telomerase works by recognizing the 3′ end of DNA, e.g., telomeres, and adding multiple telomeric repeats to its 3′ end with the catalytic protein component, e.g., hTERT, which has polymerase activity, and hTER which serves as the template for nucleotide incorporation. Of these two components of the telomerase enzyme, both the catalytic protein component and the RNA template component are activity limiting components.

Because of its role in cellular senescence and immortalization, there is much interest in the development of protocols and compositions for regulating expression of telomerase.

Relevant Literature

U.S. Patents of interest include: 6,093,809; 6,054,575; 6,007,989; 5,958,680; 5,858,777. Also of interest are WO 99/33998 and WO 99/35243. Articles of interest include: Cong et al., Hum. Mol. Genet. (1999) 8:137-142; Horikawa et al., Abstract # 1429, Scientific Proceedings, 91st Annual Meeting of American Association for Cancer Research, San Francisco, CA April 1-5, 2000; Kyo et al., Nucleic Acids Res. (2000) 28:669-677; Morgenbesser et al., The EMBO Journal (1995) 14:743-756; Takakura et al. Cancer Res. (1999) 59:551-7; and Wu et al., Nat. Genet. (1999) 21:220-224. See also GENBANK accession nos. AF114847, AF128893, AB016767, AF121948, AF097365, and AF0989756.

SUMMARY OF THE INVENTION

Methods and compositions are provided for modulating, and generally upregulating, the expression of telomerase reverse transcriptase (TERT) by blocking repression of TERT transcription, e.g., by modulating the TERT expression repressive activity of the Myc Repeat region. The subject methods and compositions find use in a variety of different applications, including the immortalization of cells, the production of reagents for use in life science research, therapeutic applications; therapeutic agent screening applications; and the like.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 provides a map of the pSSI-53 plasmid showing the insertion sites for the 2.5kbp Myc repeat region.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for modulating, and generally upregulating, the expression of telomerase reverse transcriptase (TERT) by blocking repression of TERT transcription, e.g., by modulating the TERT expression repressive activity of the Myc Repeat region. The subject methods and compositions find use in a variety of different applications, including the immortalization of cells, the production of reagents for use in life science research, therapeutic applications; therapeutic agent screening applications; and the like. In further describing the subject invention, the methods and compositions of the invention are described first in greater detail, followed by a review of the various applications in which the subject invention finds use.

Before the subject invention is described further, it is to be understood that the invention is not limited to the particular embodiments of the invention described below, as variations of the particular embodiments may be made and still fall within the scope of the appended claims. It is also to be understood that the terminology employed is for the purpose of describing particular embodiments, and is not intended to be limiting. Instead, the scope of the present invention will be established by the appended claims.

In this specification and the appended claims, the singular forms "a," "an" and "the" include plural reference unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range, and

any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods, devices and materials are now described.

METHODS

As summarized above, the subject invention provides methods and compositions for modulating expression of TERT. In the subject methods, TERT expression is modulated by modulating the TERT expression repression activity of the Myc Repeat repeat region, where modulating includes both increasing and decreasing the expression repression activity of the target repression system. As such, in certain embodiments, methods of increasing expression of TERT are provided, while in other embodiments, methods of decreasing expression of TERT are provided, where in both embodiments the modulation of expression is accomplished by modulating the repression activity of the Myc Repeat region.

Myc Repeat Region

As summarized above, the subject methods act by modulation of the TERT expression repression activity of a Myc Repeat (i.e., E-box Repeat) region, and more precisely the interaction of the Myc repeat region with one or more different transacting factors that work in concert to repress TERT

expression, where the Myc repeat region and its one or more trans activity factors are collectively referred to herein as the "target system". In certain embodiments, the target system is one that is made up of the Myc Repeat region in combination with the Myc and Mad transacting factors (both of which participate with Max for binding to the Myc Repeat region). In these embodiments, the target system is also referred to as the Myc Repeat/Myc-Mad TERT expression repression system, i.e., a Myc/Mad gene transcription regulatory system. By "Myc Repeat/Myc-Mad TERT expression repression system" or "Myc/Mad gene transcription regulatory system" is meant a regulatory system in which the expression of a certain coding sequence, e.g., a TERT coding sequence, is controlled by Myc and Mad binding (typically as dimers with Max) to an E-box repeat region of two or more E-boxes (i.e., a Myc Repeat region), where in many embodiments, the regulatory system is further characterized in that the repressive activity of Mad dominates Myc, such that when Mad binds by itself to an E-box or when both Mad and Myc (or multiple Myc's) bind to separate E-boxes within the same Myc Repeat region, transcription is repressed. In other embodiments, the target system is made up of the Myc repeat region and other transacting factors that bind to the Myc Repeat region, e.g., either the E-boxes thereof or other sequences present therein, to repress Tert expression. In many embodiments, the target system includes a set of transacting factors made up of a repressor and an activator that bind to the same site or overlapping sites, such that binding by one protein interferes with binding by the other.

Myc Repeat Region

The subject Myc Repeat/E-box repeat region component of the target repression system typically ranges in length from about 10 to about 10,000 bases, and usually ranges in length from about 50 to about 5,000 bases. In certain embodiments, the length of the subject Myc Repeat/E-box repeat region is at least about 700 bases, usually at least about 750 bases and more usually at least about 1000 bases, where the length may be as long as about 1000 bases, about 5000 bases or longer.

The subject Myc Repeat/E-box repeat region is further characterized by containing a plurality of sequence motifs known in the art as E-boxes, i.e., CACGTG. In general, the number of E-boxes present in the subject E-box repeat region may range from about 2 to about 500 or more. In certain embodiments of interest, the number of E-boxes found in the subject E-box repeat region is at least about 10, usually at least about 15 and more usually at least about 25, where the number may be about 50, about 100 or higher. In many embodiments, the number of E-boxes found in the subject E-box repeat region ranges from about 10 to 150, usually from about 25 to about 125 and is often from about 50 to about 100. The E-boxes are positioned in the E-box repeat region relatively close to each other, where the separation distance between any two given E-boxes is typically between about 25 to about 150 bases, usually between about 30 and about 130 bases and often between about 40 and about 50 bases.

In certain embodiments, the target Myc Repeat region has a nucleic acid having a sequence (at least ranging from about 10, usually from about 20 and more usually from about 25 bases in length to a length of about 50, about 100, about 200 bases or longer) found in a sequence selected from the following:

CCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGGCGCCGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGGCGCCGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGAGGCGCCGTCCCCGCGTGTC
CCTGTCACGTGCAGGGTGAGTGAGGT CCGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGGCGCCGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGGTGCCGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGAGGCCCCGTCCCCGGGTGTC
CCTGTCACATTCAGGGTGAGTGAGGCGCGGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGAGGCGCTGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGTTGCGGCCCCCGGGTGTC
CCTCTCAGGTGCAGGGTAGTGAGGC GCTGTCCCTGGGTGTC
CCTGTCTCGTGTAGGGTGAGTGAGGCTCTGTCCCCAGGTGTC
(SEQ ID NO:01)

CCTGGCTTATGCAGGGAGTG AGGCGTGGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGAGGCGTTGCCCCCAGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGGCGCGGCCCCCGGGTGTC
CCTGTCCCGTGCAGCGTGATTGAGGTGTGGCCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGGCGCCATCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGGCGTGGTCCCCGGGTGTC
CCTGTCCCGTGCAGGGTGAGTGAGGCACTGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGAGGCGCGGTCCCCGGGTGTC
CCTCTCAGGTGTAGGGTGAGTGAGGCGCGGCCCCAGGGTGTC
CCTGTCACGTGTAGGGTGAGTGAGGCACCGTCCCTGGGTGTC
CCTCCCAGGTATAGGGTGAGTGAGGCACTGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGAGGCGCGGCCCCCGGGTGTC
CCTCTCAGGTGCAGGGTGAGTGAGGCGCTGTCCCTGGGTGTC
CCTGTCTCGTGTAGGGTGAGTGAGGCTCTGTCCCCAGGTGTC
(SEQ ID NO:02)

CCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCGCCATCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCGTGGTCCCCGGGTGTC
CCTGTCCCGTGCAGGGTGAGTGA GGCACTGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCACTGTCCCCGGGTGTC
CCTCTCAGGTGTAGGGTGAGTGA GGCGCTGTCCCCGGGTGTC
CCTCTCAGGTGTAGGGTGAGTGA GGCGCGGCCCCAGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCACCGTCCCTGGGTGTC
CCTCCCAGGTATAGGGTGAGTGA GGCACTGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGTCCCCAGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCACTGTCCCCAGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGTCCCCAGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCCGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCACGGCCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGCCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCCGTCCCCGGGTGTC
TCTGTCACGTGCAGGGTGAGTGA GGCGCCGTCCCCGGGTGTC

CCTGTCACGTGCAGGGTGAGTGA GGCACGGCCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGCCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGCCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCGGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCACGGTCCCCGGGTGTC
CCTGTCACGTTCAGGGTGAGTGA GGCGCGGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAATGA GGCACTGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGAAGGCGCCGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGATTGA CGCGAGGCCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCCGTCCCCGCGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCGCCGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCGCCGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCGCCGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCGCCGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCGCCGTCCCCGGGTGTC
CCTGTCACGTGCAGGGTGAGTGA GGCCCCGTCCCCGGGTGTC
CCTGTCACGTGTAGGGTGAGTGA GGCACTGTCCCCGGG
(SEQ ID NO:03)

In other embodiments, the Myc Repeat region has a sequence that is substantially the same as, or identical to, a specific sequence identified in the immediately preceding paragraph. A given sequence is considered to be substantially similar to another sequence if the two sequences share high sequence similarity, *e.g.* at least 75% sequence identity, usually at least 90%, more usually at least 95% sequence identity. Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence. A reference sequence will usually be at least about 18 nt long, more usually at least about 30 nt long, and may extend to the complete sequence that is being compared. Algorithms for sequence analysis are known in the art, such as BLAST, described in Altschul *et al.* (1990), *J. Mol. Biol.* 215:403-10 (using default settings, i.e. parameters *w*=4 and *T*=17). Of particular interest in

certain embodiments are sequences of substantially the same length as the specific nucleic acid identified above, where by substantially the same length is meant that any difference in length does not exceed about 20 number %, usually does not exceed about 10 number % and more usually does not exceed about 5 number %; and have sequence identity to this sequence of at least about 90%, usually at least about 95% and more usually at least about 99% over the entire length of the nucleic acid.

Modulating TERT Expression

The subject invention provides methods of modulating, including both enhancing and repressing, TERT expression. As such, methods of both increasing and decreasing TERT expression are provided. In practicing the subject invention, the repressive activity of the Myc Repeat region, particularly the target system that includes the Myc repeat region, e.g. the Myc repeat/Myc-Mad TERT expression repressive system, is modulated. Included are methods of either enhancing or inhibiting the TERT expression repressive activity of this target system.

In modulating TERT expression, the interaction between the Myc Repeat region and the one or more transacting factors of the target system with which it is acting, e.g., the Myc and Mad protein components of the Myc Repeat/Myc-Mad target system, is modified in a manner that achieves the desired change in TERT expression, e.g., enhancement or reduction. This target interaction can be modified directly or indirectly. An example of direct modification of this interaction is where the binding of the transacting factor(s), e.g., the Myc and/or Mad proteins, to the target Myc Repeat region is modified by an agent that directly changes how the transacting factor(s) binds to the Myc Repeat sequence, e.g., by occupying the DNA binding sites of the transacting factor, such as the E-box binding site of the Myc and/or Mad proteins (when combined with Max), by binding to the Myc Repeat region E-boxes thereby preventing the binding of this region to the transacting factor(s), etc. An example of indirect modification is modification/modulation of the target system repressive activity via disruption of a binding interaction between the transacting factor(s) and one or more cofactors (or further upstream in the

chain of interactions) such that the repressive activity is modulated, by modification of the Myc Repeat sequence such that the repressive activity upon interaction with the transacting factors is modulated (e.g., insertion or deletion of E-boxes), etc.

Enhancing TERT Expression

Methods are provided for enhancing TERT expression. By enhancing TERT expression is meant that the expression level of the TERT coding sequence is increased by at least about 2 fold, usually by at least about 5 fold and sometimes by at least 25, 50, 100 fold and in particular about 300 fold or higher, as compared to a control, i.e., expression from an expression system that is not subjected to the methods of the present invention. Alternatively, in cases where expression of the TERT gene is so low that it is undetectable, expression of the TERT gene is considered to be enhanced if expression is increased to a level that is easily detectable.

In these methods, repression of TERT expression by the target system is inhibited. By inhibited is meant that the repressive activity of the target system with respect to TERT expression is decreased by a factor sufficient to at least provide for the desired enhanced level of TERT expression, as described above. Inhibition of the target system repression may be accomplished in a number of ways, where representative protocols for inhibiting this TERT expression repression are now provided.

One representative method of inhibiting repression of transcription is to employ double-stranded, i.e., duplex, oligonucleotide decoys for the transacting factor(s) of the target system, which bind to these transacting components and thereby prevent them from binding to their targets sequences, e.g., E-boxes, in the Myc Repeat region. These duplex oligonucleotide decoys have at least that portion of the sequence of the Myc Repeat site required to bind to the transacting factor(s), e.g., the Myc and/or Mad proteins, and thereby prevent their binding to the Myc Repeat region. In many embodiments, the subject decoy nucleic acid molecules include a sequence of nucleotides that is the same as a sequence found in SEQ ID NOs: 01 to 03. In other embodiments, the subject decoy nucleic acid

molecules include a sequence of nucleotides that is substantially the same as or identical to a sequence found in SEQ ID NOs: 01 to 03; where the terms substantially the same as and identical thereto in relation to nucleic acids are defined below. In many embodiments, the length of these duplex oligonucleotide decoys ranges from about 5 to about 5000, usually from about 5 to about 500 and more usually from about 10 to about 50 bases. In using such oligonucleotide decoys, the decoys are placed into the environment of the target system, resulting in de-repression of the transcription and expression of the TERT coding sequence. Oligonucleotide decoys and methods for their use and administration are further described in general terms in Morishita et al., Circ Res (1998) 82 (10):1023-8.

Instead of the above described decoys, other agents that disrupt binding of the target transacting factor, e.g., at least Mad in the specific Myc repeat/Myc-Mad target system described above, to the Myc Repeat region and thereby inhibit its expression repression activity may be employed. Other agents of interest include, among other types of agents, small molecules that bind to the transacting factor and inhibit its binding to the Myc repeat region. Alternatively, agents that bind to the Myc Repeat sequence and inhibit its binding to transacting factor are of interest. Alternatively, agents that disrupt protein-protein interactions of the transacting factor with cofactors, e.g., cofactor binding, and thereby inhibit the transacting factor's binding to the target Myc Repeat region and consequently inhibit expression repression are of interest.

Naturally occurring or synthetic small molecule compounds of interest include numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines,

pyrimidines, derivatives, structural analogs or combinations thereof. Such molecules may be identified, among other ways, by employing the screening protocols described below. Small molecule agents of particular interest include pyrrole-imidazole polyamides, analogous to those described in Dickinson et al., Biochemistry 1999 Aug 17;38(33):10801-7.

In yet other embodiments, expression of the transacting factor is inhibited. For example, in the specifically embodied Myc Repeat/Myc-Mad target system, inhibition of Mad expression is employed to achieve the desired increase in TERT expression, where this inhibition of Mad expression may be accomplished using any convenient means, including administration of an agent that inhibits Mad expression (e.g., antisense agents), inactivation of the Mad gene, e.g., through recombinant techniques, etc. For example, antisense molecules can be used to down-regulate expression of the target repressor protein in cells. The anti-sense reagent may be antisense oligodeoxyribonucleotides (ODN), particularly synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such anti-sense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted repressor protein, and inhibits expression of the targeted repressor protein. Antisense molecules inhibit gene expression through various mechanisms, *e.g.* by reducing the amount of mRNA available for translation, through activation of RNAse H, or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.

Antisense molecules may be produced by expression of all or a part of the target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about 7, usually at least about 12, more usually at least about 20 nucleotides in length, and not more than about 500, usually not more than about 50, more usually not more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from 7 to 8 bases in length, can be

strong and selective inhibitors of gene expression (see Wagner *et al.* (1996), *Nature Biotechnol.* 14:840-844).

A specific region or regions of the endogenous sense strand mRNA sequence is chosen to be complemented by the antisense sequence. Selection of a specific sequence for the oligonucleotide may use an empirical method, where several candidate sequences are assayed for inhibition of expression of the target gene in an *in vitro* or animal model. A combination of sequences may also be used, where several regions of the mRNA sequence are selected for antisense complementation.

Antisense oligonucleotides may be chemically synthesized by methods known in the art (see Wagner *et al.* (1993), *supra,* and Milligan *et al., supra.*) Preferred oligonucleotides are chemically modified from the native phosphodiester structure, in order to increase their intracellular stability and binding affinity. A number of such modifications have been described in the literature, which alter the chemistry of the backbone, sugars or heterocyclic bases.

Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur; phosphoroamidites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include 3′-O′-5′-S-phosphorothioate, 3′-S-5′-O-phosphorothioate, 3′-CH_2-5′-O-phosphonate and 3′-NH-5′-O-phosphoroamidate. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage. Sugar modifications are also used to enhance stability and affinity. The α-anomer of deoxyribose may be used, where the base is inverted with respect to the natural β-anomer. The 2′-OH of the ribose sugar may be altered to form 2′-O-methyl or 2′-O-allyl sugars, which provides resistance to degradation without comprising affinity. Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine; 5-methyl-2′-deoxycytidine and 5-bromo-2′-deoxycytidine for deoxycytidine. 5- propynyl-2′-deoxyuridine and 5-propynyl-2′-deoxycytidine have been shown to increase affinity and biological activity when substituted for deoxythymidine and deoxycytidine, respectively.

As an alternative to anti-sense inhibitors, catalytic nucleic acid compounds, *e.g.* ribozymes, anti-sense conjugates, *etc.* may be used to inhibit gene expression. Ribozymes may be synthesized *in vitro* and administered to the patient, or may be encoded on an expression vector, from which the ribozyme is synthesized in the targeted cell (for example, see International patent application WO 9523225, and Beigelman *et al.* (1995), *Nucl. Acids Res.* 23:4434-42). Examples of oligonucleotides with catalytic activity are described in WO 9506764. Conjugates of anti-sense ODN with a metal complex, *e.g.* terpyridylCu(II), capable of mediating mRNA hydrolysis are described in Bashkin *et al.* (1995), *Appl. Biochem. Biotechnol.* 54:43-56.

In another embodiment, the transacting factor gene, e.g., the Mad gene, is inactivated so that it no longer expresses a functional repressor protein. By inactivated is meant that the target gene, e.g., coding sequence and/or regulatory elements thereof, is genetically modified so that it no longer expresses functional repressor protein. The alteration or mutation may take a number of different forms, e.g., through deletion of one or more nucleotide residues in the repressor region, through exchange of one or more nucleotide residues in the repressor region, and the like. One means of making such alterations in the coding sequence is by homologous recombination. Methods for generating targeted gene modifications through homologous recombination are known in the art, including those described in: U.S. Patent Nos. 6,074,853; 5,998,209 ; 5,998,144; 5,948,653; 5,925,544; 5,830,698; 5,780,296; 5,776,744; 5,721,367; 5,614,396; 5,612,205; the disclosures of which are herein incorporated by reference.

The above described methods of enhancing TERT expression find use in a number of different applications. In many applications, the subject methods and compositions are employed to enhance TERT expression in a cell that endogenously comprises a TERT gene, e.g. for enhancing expression of hTERT in a normal human cell in which TERT expression is repressed. The target cell of these applications is, in many instances, a normal cell, e.g. a somatic cell. Expression of the TERT gene is considered to be enhanced if, consistent with the above description, expression is increased by at least about 2 fold, usually at least about 5 fold and often 25, 50, 100 fold, 300 fold or higher, as compared to a control, e.g., an otherwise identical cell not subjected

to the subject methods, or becomes detectable from an initially undetectable state, as described above.

A more specific application in which the subject methods find use is to increase the proliferative capacity of a cell. The term "proliferative capacity" as used herein refers to the number of divisions that a cell can undergo, and preferably to the ability of the target cell to continue to divide where the daughter cells of such divisions are not transformed, i.e., they maintain normal response to growth and cell cycle regulation. The subject methods typically result in an increase in proliferative capacity of at least about 1.2 - 2 fold, usually at least about 5 fold and often at least about 10, 20, 50 fold or even higher, compared to a control. As such, yet another more specific application in which the subject methods find use is in the delay of the occurrence of cellular senescence. By practicing the subject methods, the onset of cellular senescence may be delayed by a factor of at least about 1.2 - 2 fold, usually at least about 5 fold and often at least about 10, 20, 50 fold or even higher, compared to a control.

Methods of Inhibiting TERT Expression

As mentioned above, also provided are methods for inhibiting TERT expression, e.g., by enhancing repression of TERT expression by the target system and thereby inhibiting TERT expression. In such methods, the amount and/or activity of transacting factor, e.g., Mad, is increased so as to enhance repression of TERT expression by the target system. A variety of different protocols may be employed to achieve this result, including administration of an effective amount of the transacting factor or analog/mimetic thereof, an agent that enhances expression of the transacting factor or an agent that enhances the activity of the transacting factor.

As such, nucleic acid compositions that encode the transacting factor find use in situations where one wishes to enhance the activity of transacting factor in a host. The repressor protein genes, gene fragments, or the encoded proteins or protein fragments are useful in gene therapy to treat disorders in which inhibition of TERT expression is desired, including those applications described in greater detail below. Expression vectors may be used to

introduce the gene into a cell. Such vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences. Transcription cassettes may be prepared comprising a transcription initiation region, the target gene or fragment thereof, and a transcriptional termination region. The transcription cassettes may be introduced into a variety of vectors, *e.g.* plasmid; retrovirus, *e.g.* lentivirus; adenovirus; and the like, where the vectors are able to transiently or stably be maintained in the cells, usually for a period of at least about one day, more usually for a period of at least about several days to several weeks.

The gene or protein may be introduced into tissues or host cells by any number of routes, including viral infection, microinjection, or fusion of vesicles. Jet injection may also be used for intramuscular administration, as described by Furth et al. (1992), Anal Biochem 205:365-368. The DNA may be coated onto gold microparticles, and delivered intradermally by a particle bombardment device, or "gene gun" as described in the literature (see, for example, Tang et al. (1992), Nature 356:152-154), where gold microprojectiles are coated with the DNA, then bombarded into skin cells.

Therapeutic Applications of TERT Expression Modulation

The methods find use in a variety of therapeutic applications in which it is desired to modulate, e.g., increase or decrease, TERT expression in a target cell or collection of cells, where the collection of cells may be a whole animal or portion thereof, e.g., tissue, organ, etc. As such, the target cell(s) may be a host animal or portion thereof, or may be a therapeutic cell (or cells) which is to be introduced into a multicellular organism, e.g., a cell employed in gene therapy. In such methods, an effective amount of an active agent that modulates TERT expression, e.g., enhances or decreases TERT expression as desired, is administered to the target cell or cells, e.g., by contacting the cells with the agent, by administering the agent to the animal, etc. By effective amount is meant a dosage sufficient to modulate TERT expression in the target cell(s), as desired.

In the subject methods, the active agent(s) may be administered to the targeted cells using any convenient means capable of resulting in the desired

enhancement of TERT expression. Thus, the agent can be incorporated into a variety of formulations for therapeutic administration. More particularly, the agents of the present invention can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments (e.g., skin creams), solutions, suppositories, injections, inhalants and aerosols. As such, administration of the agents can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, etc., administration.

In pharmaceutical dosage forms, the agents may be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

For oral preparations, the agents can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

The agents can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

The agents can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

Furthermore, the agents can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The compounds of the present invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may comprise the inhibitor(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

Where the agent is a polypeptide, polynucleotide, analog or mimetic thereof, e.g. oligonucleotide decoy, it may be introduced into tissues or host cells by any number of routes, including viral infection, microinjection, or fusion of vesicles. Jet injection may also be used for intramuscular administration, as described by Furth *et al.* (1992), *Anal Biochem* 205:365-368. The DNA may be coated onto gold microparticles, and delivered intradermally by a particle bombardment device, or "gene gun" as described in the literature (see, for example, Tang *et al.* (1992), *Nature* 356:152-154), where gold microprojectiles

are coated with the DNA, then bombarded into skin cells. For nucleic acid therapeutic agents, a number of different delivery vehicles find use, including viral and non-viral vector systems, as are known in the art.

Those of skill in the art will readily appreciate that dose levels can vary as a function of the specific compound, the nature of the delivery vehicle, and the like. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

The subject methods find use in the treatment of a variety of different conditions in which the enhancement of TERT expression in the host is desired. By treatment is meant that at least an amelioration of the symptoms associated with the condition afflicting the host is achieved, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, *e.g.* symptom (such as inflammation), associated with the condition being treated. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, *e.g.* prevented from happening, or stopped, *e.g.* terminated, such that the host no longer suffers from the condition, or at least the symptoms that characterize the condition.

A variety of hosts are treatable according to the subject methods. Generally such hosts are "mammals" or "mammalian," where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (*e.g.*, dogs and cats), rodentia (*e.g.*, mice, guinea pigs, and rats), and primates (*e.g.*, humans, chimpanzees, and monkeys). In many embodiments, the hosts will be humans.

As indicated above, the subject invention provides methods of treating disease conditions resulting from a lack of TERT expression and methods of treating disease conditions resulting from unwanted TERT expression. Representative disease conditions for each category are now described in greater detail separately.

Treatment of Disease Conditions by Increasing TERT Expression

One representative disease condition that may be treated according to the subject invention is Progeria, or Hutchinson-Gilford syndrome. This

condition is a disease of shortened telomeres for which no known cure exists. It afflicts children, who seldom live past their early twenties. In many ways progeria parallels aging itself. However, these children are born with short telomeres. Their telomeres don't shorten at a faster rate; they are just short to begin with. The subject methods can be used in such conditions to further delay natural telomeric shortening and/or increase telomeric length, thereby treating this condition.

Another specific disease condition in which the subject methods find use is in immune senescence. The effectiveness of the immune system decreases with age. Part of this decline is due to fewer T-lymphocytes in the system, a result of lost replicative capacity. Many of the remaining T-lymphocytes experience loss of function as their telomeres shorten and they approach senescence. The subject methods can be employed to inhibit immune senescence due to telomere loss. Because hosts with aging immune systems are at greater risk of developing pneumonia, cellulitis, influenza, and many other infections, the subject methods reduce morbidity and mortality due to infections.

The subject methods also find use in AIDS therapy. HIV, the virus that causes AIDS, invades white blood cells, particularly CD4 lymphocyte cells, and causes them to reproduce high numbers of the HIV virus, ultimately killing cells. In response to the loss of immune cells (typically about a billion per day), the body produces more CD8 cells to be able to suppress infection. This rapid cell division accelerates telomere shortening, ultimately hastening immune senescence of the CD8 cells. Anti-retroviral therapies have successfully restored the immune systems of AIDS patients, but survival depends upon the remaining fraction of the patient's aged T-cells. Once shortened, telomere length has not been naturally restored within cells. The subject methods can be employed to restore this length and/or prevent further shortening. As such the subject methods can spare telomeres and is useful in conjunction with the anti-retroviral treatments currently available for HIV.

Yet another type of disease condition in which the subject methods find use is cardiovascular disease. The subject methods can be employed to extend telomere length and replicative capacity of endothelial cells lining blood vessel walls (DeBono, Heart 80:110-1, 1998). Endothelial cells form the inner

lining of blood vessels and divide and replace themselves in response to stress. Stresses include high blood pressure, excess cholesterol, inflammation, and flow stresses at forks in vessels. As endothelial cells age and can no longer divide sufficiently to replace lost cells, areas under the endothelial layer become exposed. Exposure of the underlying vessel wall increases inflammation, the growth of smooth muscle cells, and the deposition of cholesterol. As a result, the vessel narrows and becomes scarred and irregular, which contributes to even more stress on the vessel (Cooper, Cooke and Dzau, J Gerontol Biol Sci **49**: 191-6, 1994). Aging endothelial cells also produce altered amounts of trophic factors (hormones that affect the activity of neighboring cells). These too contribute to increased clotting, proliferation of smooth muscle cells, invasion by white blood cells, accumulation of cholesterol, and other changes, many of which lead to plaque formation and clinical cardiovascular disease (*Ibid.*). By extending endothelial cell telomeres, the subject methods can be employed to combat the stresses contributing to vessel disease. Many heart attacks may be prevented if endothelial cells were enabled to continue to divide normally and better maintain cardiac vessels. The occurrence of strokes caused by the aging of brain blood vessels may also be significantly reduced by employing the subject methods to help endothelial cells in the brain blood vessels to continue to divide and perform their intended function.

The subject methods also find use in skin rejuvenation. The skin is the first line of defense of the immune system and shows the most visible signs of aging (West, Arch Dermatol 130(1):87-95, 1994). As skin ages, it thins, develops wrinkles, discolors, and heals poorly. Skin cells divide quickly in response to stress and trauma; but, over time, there are fewer and fewer actively dividing skin cells. Compounding the loss of replicative capacity in aging skin is a corresponding loss of support tissues. The number of blood vessels in the skin decreases with age, reducing the nutrients that reach the skin. Also, aged immune cells less effectively fight infection. Nerve cells have fewer branches, slowing the response to pain and increasing the chance of trauma. In aged skin, there are also fewer fat cells, increasing susceptibility to cold and temperature changes. Old skin cells respond more slowly and less accurately to external signals. They produce less vitamin D, collagen, and

elastin, allowing the extracellular matrix to deteriorate. As skin thins and loses pigment with age, more ultraviolet light penetrates and damages skin. To repair the increasing ultraviolet damage, skin cells need to divide to replace damaged cells, but aged skin cells have shorter telomeres and are less capable of dividing (Fossel, REVERSING HUMAN AGING. William Morrow & Company, New York City, 1996).

By practicing the subject methods, e.g., via administration of an active agent topically, one can extend telomere length, and slow the downward spiral that skin experiences with age. Such a product not only helps protect a person against the impairments of aging skin; it also permits rejuvenated skin cells to restore youthful immune resistance and appearance. The subject methods can be used for both medical and cosmetic skin rejuvenation applications.

Yet another disease condition in which the subject methods find use in the treatment of osteoporosis. Two types of cells interplay in osteoporosis: osteoblasts make bone and osteoclasts destroy it. Normally, the two are in balance and maintain a constant turnover of highly structured bone. In youth, bones are resilient, harder to break, and heal quickly. In old age, bones are brittle, break easily, and heal slowly and often improperly. Bone loss has been postulated to occur because aged osteoblasts, having lost much of their replicative capacity, cannot continue to divide at the rate necessary to maintain balance (Hazzard et al. PRINCIPLES OF GERIATRIC MEDICINE AND GERONTOLOGY, 2d ed. McGraw-Hill, New York City, 1994). The subject methods can be employed to lengthen telomeres of osteoblast and osteoclast stem cells, thereby encouraging bone replacement and proper remodeling and reinforcement. The resultant stronger bone improves the quality of life for the many sufferers of osteoporosis and provides savings from fewer fracture treatments. The subject methods are generally part of a comprehensive treatment regime that also includes calcium, estrogen, and exercise.

Additional disease conditions in which the subject methods find use are described in WO 99/35243, the disclosures of which are herein incorporated by reference.

In addition to the above described methods, the subject methods can also be used to extend the lifetime of a mammal. By extend the lifetime is

meant to increase the time during which the animal is alive, where the increase is generally at least 1 %, usually at least 5% and more usually at least about 10 %, as compared to a control.

As indicated above, instead of a multicellular animal, the target may be a cell or population of cells which are treated according to the subject methods and then introduced into a multicellular organism for therapeutic effect. For example, the subject methods may be employed in bone marrow transplants for the treatment of cancer and skin grafts for burn victims. In these cases, cells are isolated from a human donor and then cultured for transplantation back into human recipients. During the cell culturing, the cells normally age and senesce, decreasing their useful lifespans. Bone marrow cells, for instance, lose approximately 40 % of their replicative capacity during culturing. This problem is aggravated when the cells are first genetically engineered (Decary, Mouly et al. Hum Gene Ther 7(11): 1347-50, 1996). In such cases, the therapeutic cells must be expanded from a single engineered cell. By the time there are sufficient cells for transplantation, the cells have undergone the equivalent of 50 years of aging (Decary, Mouly et al. Hum Gene Ther 8(12): 1429-38, 1997). Use of the subject methods spares the replicative capacity of bone marrow cells and skin cells during culturing and expansion and thus significantly improves the survival and effectiveness of bone marrow and skin cell transplants. Any transplantation technology requiring cell culturing can benefit from the subject methods, including ex vivo gene therapy applications in which cells are cultured outside of the animal and then administered to the animal, as described in U.S. Patent Nos. 6,068,837; 6,027,488; 5,824,655; 5,821,235; 5,770,580; 5,756,283; 5,665,350; the disclosures of which are herein incorporated by reference.

Treatment of Disease Conditions by Decreasing TERT Expression

As summarized above, also provided are methods for enhancing repression of TERT expression, where by enhancement of TERT expression repression is meant a decrease in TERT expression by a factor of at least about 2 fold, usually at least about 5 fold and more usually at least about 10 fold, as compared to a control. Methods for enhancing Myc Repeat region

mediated repression of TERT expression find use in, among other applications, the treatment of cellular proliferative disease conditions, particularly abnormal cellular proliferative disease conditions, including, but not limited to, neoplastic disease conditions, e.g., cancer. In such applications, an effective amount of an active agent, e.g., a transacting factor, analog or mimetic thereof, (such as Mad) a vector encoding the same or active fragment thereof, an agent that enhances endogenous transacting factor activity, an agent that enhances expression of the transacting factor, etc., is administered to the subject in need thereof. Treatment is used broadly as defined above, e.g., to include at least an amelioration in one or more of the symptoms of the disease, as well as a complete cessation thereof, as well as a reversal and/or complete removal of the disease condition, e.g., cure. Methods of treating disease conditions resulting from unwanted TERT expression, such as cancer and other diseases characterized by the presence of unwanted cellular proliferation, are described in, for example, U.S. Patent Nos. 5,645,986; 5,656,638; 5,703,116; 5,760,062; 5,767,278; 5,770,613; and 5,863,936; the disclosures of which are herein incorporated by reference.

NUCLEIC ACID COMPOSITIONS

Also provided by the subject invention are nucleic acid compositions, where the compositions are present in other than their natural environment, e.g., are isolated, recombinant, etc., that include a Myc Repeat domain/region, as described above. In other embodiments, the subject nucleic acids have a sequence that is substantially the same as, or identical to, the Myc Repeat sequences as described above, e.g., SEQ ID NOs: 01 to 03. A given sequence is considered to be substantially similar to this particular sequence if it shares high sequence similarity with the above described specific sequences, *e.g.* at least 75% sequence identity, usually at least 90%, more usually at least 95% sequence identity with the above specific sequences. Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence. A reference sequence will usually be at least about 18 nt long, more usually at least about 30 nt long, and may extend to the complete sequence that is being compared. Algorithms for sequence

analysis are known in the art, such as BLAST, described in Altschul *et al.* (1990), *J. Mol. Biol.* 215:403-10 (using default settings, i.e. parameters *w*=4 and *T*=17). Of particular interest in certain embodiments are nucleic acids of substantially the same length as the specific nucleic acid identified above, where by substantially the same length is meant that any difference in length does not exceed about 20 number %, usually does not exceed about 10 number % and more usually does not exceed about 5 number %; and have sequence identity to this sequence of at least about 90%, usually at least about 95% and more usually at least about 99% over the entire length of the nucleic acid.

Also provided are nucleic acids that hybridize to the above described nucleic acid under stringent conditions. An example of stringent hybridization conditions is hybridization at 50°C or higher and 0.1×SSC (15 mM sodium chloride/1.5 mM sodium citrate). Another example of stringent hybridization conditions is overnight incubation at 42°C in a solution: 50 % formamide, 5 × SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5 × Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1 × SSC at about 65°C. Stringent hybridization conditions are hybridization conditions that are at least as stringent as the above representative conditions, where conditions are considered to be at least as stringent if they are at least about 80% as stringent, typically at least about 90% as stringent as the above specific stringent conditions. Other stringent hybridization conditions are known in the art and may also be employed to identify nucleic acids of this particular embodiment of the invention.

In many embodiments, the above described nucleic acid compositions include the Myc repeat domain region but do not include all of the components of the TERT genomic sequence, e.g., all of the other intron/exon regions of the TERT genomic sequence. In these embodiments, the subject nucleic acids include no more than about 90 number %, usually no more than about 80 number % and more usually no more than about 75 number %, where in many embodiments the subject nucleic acids include less than about 50 number %, sometimes less than about 40 number % and sometimes less than about 25 number % of the total sequence of the TERT genomic sequence. In certain

embodiments, the length of the subject nucleic acids ranges from about 5 to about 5000 bases, sometimes from about 10 to about 2500 bases and usually from about 10 to about 1000 bases.

The above described nucleic acid compositions find use in a variety of different applications, including the preparation of constructs, e.g., vectors, expression systems, etc., as described more fully below, the preparation of probes for the Myc Repeat sequence in non-human animals, i.e., non-human Myc Repeat homologs, and the like. Where the subject nucleic acids are employed as probes, a fragment of the provided nucleic acid may be used as a hybridization probe against a genomic library from the target organism of interest, where low stringency conditions are used. The probe may be a large or small fragment, generally ranging in length from about 10 to 100 nt, usually from about 15 to 50 nt. Nucleic acids having sequence similarity are detected by hybridization under low stringency conditions, for example, at 50°C and 6×SSC (0.9 M sodium chloride/0.09 M sodium citrate) and remain bound when subjected to washing at 55°C in 1×SSC (0.15 M sodium chloride/0.015 M sodium citrate). Sequence identity may be determined by hybridization under stringent conditions, for example, at 50°C or higher and 0.1×SSC (15 mM sodium chloride/01.5 mM sodium citrate). Nucleic acids having a region of substantial identity to the provided nucleic acid sequences bind to the provided sequences under stringent hybridization conditions. By using probes, particularly labeled probes of DNA sequences, one can isolate homologous or related sequences.

The subject nucleic acids are isolated and obtained in substantial purity, generally as other than an intact chromosome. As such, they are present in other than their naturally occurring environment. Usually, the DNA will be obtained substantially free of other nucleic acid sequences that do not include a Myc repeat region or fragment thereof, generally being at least about 50%, usually at least about 90% pure and are typically "recombinant", *i.e.* flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome.

The subject nucleic acids may be produced using any convenient protocol, including synthetic protocols, e.g., those where the nucleic acid is synthesized by a sequential monomeric approach (e.g., via phosphoramidite

chemistry); where subparts of the nucleic acid are so synthesized and then assembled or concatamerized into the final nucleic acid, and the like. Where the nucleic acid of interest has a sequence that occurs in nature, the nucleic acid may be retrieved, isolated, amplified etc., from a natural source using conventional molecular biology protocols.

Also provided are nucleic acid compositions that include a modified or altered Myc Repeat region, e.g., where the site includes one or more deletions or substitutions as compared to the above specific Myc Repeat region. The subject nucleic acids of this embodiment that include a deletion (or substitution) in all or a portion of the Myc repeat may be present in the genome of a cell or animal of interest, e.g., as a "knockout" deletion in a transgenic cell or animal, where the cell or animal initially has this region, or may be present in an isolated form. A "knockout" animal could be produced from an animal that originally has the subject Myc Repeat using the sequences flanking specific Myc Repeat regions described here and the basic "knockout" technology known to those skilled in the art e.g. see U.S. Patent 5,464,764 to Capecchi.

Also provided are constructs comprising the subject nucleic acid compositions, e.g., those that include the Myc Repeat or those that include a deletion in the Myc Repeat region, inserted into a vector, where such constructs may be used for a number of different applications, including propagation, screening, genome alteration, and the like, as described in greater detail below. Constructs made up of viral and non-viral vector sequences may be prepared and used, including plasmids, as desired. The choice of vector will depend on the particular application in which the nucleic acid is to be employed. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture, e.g., for use in screening assays. Still other vectors are suitable for transfer and expression in cells in a whole animal or person. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially. To prepare the constructs, the partial or full-length nucleic acid is inserted into a vector typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence can be inserted by homologous

recombination in vivo. Typically this is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence. Regions of homology are added by ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence, for example. Additional examples of nucleic acid compositions that include the Myc Repeat are polymers, e.g. a double stranded DNA molecules, that mimic the Myc repeat site as described above.

Also provided are expression cassettes, vectors or systems that find use in, among other applications, screening for agents that modulate, e.g., inhibit or enhance the repressive activity of the region, as described in greater detail below; and/or to provide for expression of proteins under the control of the expression regulation mechanism of the TERT gene. By expression cassette or system is meant a nucleic acid that includes a sequence encoding a peptide or protein of interest, i.e., a coding sequence, operably linked to a promoter sequence, where by operably linked is meant that expression of the coding sequence is under the control of the promoter sequence. The expression systems and cassettes of the subject invention comprise a Myc Repeat region that, in the presence of the other target system components, e.g., the Mad/Myc components, of the target expression repression system, can modulate expression of a coding sequence to which it is operably linked.

As indicated above, expression systems comprising the subject regions find use in applications where it is desired to control expression of a particular coding sequence using the TERT transcriptional mechanism. In such applications, the expression system further includes the coding sequence of interest operably linked to the Myc Repeat element. The expression system is then employed in an appropriate environment to provide expression or non-expression of the protein, as desired, e.g., in an environment in which telomerase is expressed, e.g., a Hela cell, or in an environment in which telomerase is not expressed, e.g., an MRC5 cell. Alternatively, the expression system may be used in an environment in which telomerase expression is inducible, e.g., by adding to the system an additional agent that turns on telomerase expression.

The above applications of the subject nucleic acid compositions are merely representative of the diverse applications in which the subject nucleic acid compositions find use.

GENERATION OF ANTIBODIES

Also provided are methods of generating antibodies, e.g., monoclonal antibodies. In one embodiment, the blocking or inhibition, either directly or indirectly as described above, of the TERT expression repressive activity of the Myc Repeat region is used to immortalize cells in culture, e.g., by enhancing telomerase expression. Exemplary of cells that may be used for this purpose are non-transformed antibody producing cells, *e.g.* B cells and plasma cells which may be isolated and identified for their ability to produce a desired antibody using known technology as, for example, taught in U.S. patent 5,627,052. These cells may either secrete antibodies (antibody-secreting cells) or maintain antibodies on the surface of the cell without secretion into the cellular environment. Such cells have a limited lifespan in culture, and are usefully immortalized by upregulating expression of telomerase using the methods of the present invention.

Because the above described methods are methods of increasing expression of TERT and therefore increasing the proliferative capacity and/or delaying the onset of senescence in a cell, they find applications in the production of a range of reagents, typically cellular or animal reagents. For example, the subject methods may be employed to increase proliferation capacity, delay senescence and/or extend the lifetimes of cultured cells. Cultured cell populations having enhanced TERT expression are produced using any of the protocols as described above, including by contact with an agent that inhibits repressor region transcription repression and/or modification of the repressor region in a manner such that it no longer represses TERT coding sequence transcription, etc.

The subject methods find use in the generation of monoclonal antibodies. An antibody-forming cell may be identified among antibody-forming cells obtained from an animal which has either been immunized with a selected substance, or which has developed an immune response to an antigen as a result of disease. Animals may be immunized with a selected antigen using any of the techniques well known in the art suitable for generating an immune response. Antigens may include any substance to which an antibody may be made, including, among others, proteins, carbohydrates, inorganic or organic molecules, and transition state analogs that resemble intermediates in an enzymatic process. Suitable antigens include, among others, biologically active proteins, hormones, cytokines, and their cell surface receptors, bacterial or parasitic cell membrane or purified components thereof, and viral antigens.

As will be appreciated by one of ordinary skill in the art, antigens which are of low immunogenicity may be accompanied with an adjuvant or hapten in order to increase the immune response (for example, complete or incomplete Freund's adjuvant) or with a carrier such as keyhole limpet hemocyanin (KLH).

Procedures for immunizing animals are well known in the art. Briefly, animals are injected with the selected antigen against which it is desired to raise antibodies. The selected antigen may be accompanied by an adjuvant or hapten, as discussed above, in order to further increase the immune response. Usually the substance is injected into the peritoneal cavity, beneath the skin, or into the muscles or bloodstream. The injection is repeated at varying intervals and the immune response is usually monitored by detecting antibodies in the serum using an appropriate assay that detects the properties of the desired antibody. Large numbers of antibody-forming cells can be found in the spleen and lymph node of the immunized animal. Thus, once an immune response has been generated, the animal is sacrificed, the spleen and lymph nodes are removed, and a single cell suspension is prepared using techniques well known in the art.

Antibody-forming cells may also be obtained from a subject which has generated the cells during the course of a selected disease. For instance, antibody-forming cells from a human with a disease of unknown cause, such as rheumatoid arthritis, may be obtained and used in an effort to identify

antibodies which have an effect on the disease process or which may lead to identification of an etiological agent or body component that is involved in the cause of the disease. Similarly, antibody-forming cells may be obtained from subjects with disease due to known etiological agents such as malaria or AIDS. These antibody forming cells may be derived from the blood or lymph nodes, as well as from other diseased or normal tissues. Antibody-forming cells may be prepared from blood collected with an anticoagulant such as heparin or EDTA. The antibody-forming cells may be further separated from erythrocytes and polymorphs using standard procedures such as centrifugation with Ficoll-Hypaque (Pharmacia, Uppsula, Sweden). Antibody-forming cells may also be prepared from solid tissues such as lymph nodes or tumors by dissociation with enzymes such as collagenase and trypsin in the presence of EDTA.

Antibody-forming cells may also be obtained by culture techniques such as in vitro immunization. Briefly, a source of antibody-forming cells, such as a suspension of spleen or lymph node cells, or peripheral blood mononuclear cells are cultured in medium such as RPMI 1640 with 10% fetal bovine serum and a source of the substance against which it is desired to develop antibodies. This medium may be additionally supplemented with amounts of substances known to enhance antibody-forming cell activation and proliferation such as lipopolysaccharide or its derivatives or other bacterial adjuvants or cytokines such as IL-1, IL-2, IL-4, IL-5, IL-6, GM-CSF, and IFN-.gamma.. To enhance immunogenicity, the selected antigen may be coupled to the surface of cells, for example, spleen cells, by conventional techniques such as the use of biotin/avidin as described below.

Antibody-forming cells may be enriched by methods based upon the size or density of the antibody-forming cells relative to other cells. Gradients of varying density of solutions of bovine serum albumin can also be used to separate cells according to density. The fraction that is most enriched for desired antibody-forming cells can be determined in a preliminary procedure using the appropriate indicator system in order to establish the antibody-forming cells.

The identification and culture of antibody producing cells of interest is followed by enhancement of TERT expression in these cells by the subject methods, thereby avoiding the need for the immortalization/fusing step employed in traditional hybridoma manufacture protocols. In such methods, the first step is immunization of the host animal with an immunogen, typically a polypeptide, where the polypeptide will preferably be in substantially pure form, comprising less than about 1% contaminant. The immunogen may comprise the complete protein, fragments or derivatives thereof. To increase the immune response of the host animal, the protein may be combined with an adjuvant, where suitable adjuvants include alum, dextran sulfate, large polymeric anions, oil & water emulsions, e.g. Freund's adjuvant, Freund's complete adjuvant, and the like. The protein may also be conjugated to synthetic carrier proteins or synthetic antigens. A variety of hosts may be immunized to produce the subject antibodies. Such hosts include rabbits, guinea pigs, rodents (e.g. mice, rats), sheep, goats, and the like. The protein is administered to the host, usually intradermally, with an initial dosage followed by one or more, usually at least two, additional booster dosages. Following immunization, generally, the spleen and/or lymph nodes of an immunized host animal provide a source of plasma cells. The plasma cells are treated according to the subject invention to enhance TERT expression and thereby, increase the proliferative capacity and/or delay senescence to produce "pseudo" immortalized cells. Culture supernatant from individual cells is then screened using standard techniques to identify those producing antibodies with the desired specificity. Suitable animals for production of monoclonal antibodies to a human protein include mouse, rat, hamster, *etc.* To raise antibodies against the mouse protein, the animal will generally be a hamster, guinea pig, rabbit, etc. The antibody may be purified from the cell supernatants or ascites fluid by conventional techniques, *e.g.* affinity chromatography using RFLAT-1protein bound to an insoluble support, protein A sepharose, *etc.*

In an analogous fashion, the subject methods are employed to enhance TERT expression in non-human animals, e.g., non-human animals employed in laboratory research. Using the subject methods with such animals can provide a number of advantages, including extending the lifetime of difficult

and/or expensive to produce transgenic animals. As with the above described cells and cultures thereof, the expression of TERT in the target animals may be enhanced using a number of different protocols, including the administration of an agent that inhibits the TERT expression repression of the target system. The subject methods may be used with a number of different types of animals, where animals of particular interest include mammals, e.g., rodents such as mice and rats, cats, dogs, sheep, rabbits, pigs, cows, horses, and non-human primates, e.g. monkeys, baboons, etc.

SCREENING ASSAYS

Also provided by the subject invention are screening protocols and assays for identifying agents that modulate, e.g., inhibit or enhance, Myc Repeat region repression of TERT transcription. The screening methods include assays that provide for qualitative/quantitative measurements of TERT promoter controlled expression, e.g., of a coding sequence for a marker or reporter gene, in the presence of a particular candidate therapeutic agent. Assays of interest include assays that measure the TERT promoter controlled expression of a reporter gene (i.e. coding sequence, e.g., luciferase, SEAP, etc.) in the presence and absence of a candidate inhibitor agent, e.g., the expression of the reporter gene in the presence or absence of a candidate agent. The screening method may be an *in vitro* or *in vivo* format, where both formats are readily developed by those of skill in the art. Whether the format is *in vivo* or *in vitro*, an expression system, e.g., a plasmid, that includes a Myc Repeat region and a reporter coding sequence all operably linked is combined with the candidate agent in an environment in which, in the absence of the candidate agent, expression of the coding sequence is repressed, e.g., in the presence of a combination of Myc and Mad that causes TERT promoter repression. The conditions may be set up *in vitro* by combining the various required components in an aqueous medium, or the assay may be carried out *in vivo*, e.g., in a cell that normally lacks telomerase activity, e.g., an MRC5 cell, etc.

In certain embodiments, the screening assays are screening protocols and assays for identifying agents that modulate a Myc/Mad transcription

regulatory system, e.g., inhibit or enhance, TERT transcription. By Myc/Mad gene transcription regulatory system is meant a regulatory system in which the expression of a certain coding sequence is controlled by Myc and Mad binding to an E-box repeat region of two or more E-boxes, where in many embodiments, the regulatory system is further characterized in that the repressive activity of Mad dominates Myc, such that when Mad binds by itself to an E-box or when both Mad and Myc (or multiple Myc's) bind to separate E-boxes within the same Myc Repeat region, transcription is repressed. The compositions may be naturally occurring or synthetic, where when they are naturally occurring they are present in other than their natural environment, e.g., are isolated, recombinant, etc. In these embodiments, the Myc Repeat region may be a component of the screening assay, as described above. Alternatively, a nucleic acid component that mimics this region may be employed. For example, a nucleic acid that has an E-box repeat region may be employed, where the E-box repeat region may range in length from about 10 to about 10,000 bases, and usually ranges in length from about 50 to about 5,000 bases. In certain embodiments, the length of the subject E-box repeat region is at least about 700 bases, usually at least about 750 bases and more usually at least about 1000 bases, where the length may be as long as 1000 bases, 5000 bases or longer. The subject E-box repeat region is further characterized by containing a plurality of sequence motifs known in the art as E-boxes, i.e., CACGTG. In general, the number of E-boxes present in the subject E-box repeat region may range from about 2 to about 500 or more. In certain embodiments of interest, the number of E-boxes found in the subject E-box repeat region is at least about 10, usually at least about 15 and more usually at least about 25, where the number may be 50, 100 or higher. In many embodiments, the number of E-boxes found in the subject E-box repeat region ranges from about 10 to 150, usually from about 25 to 125 and is often from about 50 to 100. The E-boxes are positioned in the E-box repeat region relatively close to each other, where the separation distance between any two given E-boxes is typically between about 25 to about 150 bases, usually between about 30 and about 130 bases and often between about 40 and about 50 bases.

A variety of different candidate agents may be screened by the above methods. Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, *etc.* to produce structural analogs.

Agents identified in the above screening assays that inhibit repression of TERT transcription find use in the methods described above, e.g., in the enhancement of TERT expression. Alternatively, agents identified in the above screening assays that enhance repression find use in applications where inhibition of TERT expression is desired, e.g., in the treatment of disease conditions characterized by the presence of unwanted TERT expression, such as cancer and other diseases characterized by the presence of unwanted cellular proliferation, where such methods are described in, for example, U.S. Patent Nos. 5,645,986; 5,656,638; 5,703,116; 5,760,062; 5,767,278;

5,770,613; and 5,863,936; the disclosures of which are herein incorporated by reference.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

A region of the TERT genomic DNA labeled the Myc Repeat region was identified in the course of performing Southern Blots on various cell lines. The most common, and putative natural size of the Myc Repeat region is around 4500 bases. A smaller version measured to be 2500 bases was also identified. The sequence of this 2500 base Myc repeat region shows that within 1500 bases there are 31 E-Boxes. As such, the natural 4500 base Myc Repeat is expected to have approximately 100 E-Boxes and the smaller 2500 base Myc Repeat has approximately 50 E-Boxes.

The 2500 base Myc Repeat was inserted into the plasmid pSSI-53 (Shown in Fig. 1) to test its affect on expression of the telomerase minimal promoter. The 2500 base Myc Repeat was placed into two different sites upstream of the minimal promoter (See Fig. 1), an XHO1 site and a NOT1 site. The NOT1 site was used because it is upstream of a transcription blocker and, if increased expression of the telomerase promoter using these constructions were observed, it would be known that the increase in expression was not due to promoter activity within the Myc Repeat, but was, in fact, due to activation of the telomerase promoter. On the other hand, increased expression due to the Myc repeat inserted into the XHO1 site could not be distinguished as such. However, in all cases, NOT1 insertion, XHO1 insertion, and both orientations of the Myc repeat into each site, showed a 5-10 fold decrease in expression.

The above results indicate that the Myc repeat region or a portion thereof , e.g., a region of neighboring E boxes, interacts with one or more transacting factors, e.g., E-box binding proteins such as Myc and/or Mad or Myc and/or Mad like proteins, to repress Tert expression. The above results

also indicate that, in certain embodiments, upon dual binding of Myc and Mad to neighboring E-box sites, Mad dominates to result in transcription repression

It is evident from the above results and discussion that the subject invention provides important new nucleic acid compositions that find use in a variety of applications, including the establishment of expression systems that exploit Myc/Mad transcription regulatory systems, such as the regulatory mechanism of the TERT gene, and the establishment of screening assays for agents that enhance TERT expression. In addition, the subject invention provides methods of enhancing TERT expression in a cellular or animal host, which methods find use in a variety of applications, including the production of scientific research reagents and therapeutic treatment applications. It is evident from the above results and discussion that the subject invention also provides important new nucleic acid compositions that find use in a variety of applications, including the establishment of expression systems that exploit the regulatory mechanism of the TERT gene and the establishment of screening assays for agents that enhance TERT expression. Accordingly, the subject invention represents significant contribution to the art.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of modulating expression of TERT from a TERT expression system that includes a Myc Repeat region, said method comprising:
modulating TERT transcription repression by said Myc Repeat region.

2. The method according to Claim 1, wherein said expression system is present in a cell-free environment.

3. The method according to Claim 1, wherein said expression system is present inside of a cell.

4. The method according to Claim 1, wherein said expression system comprises a TERT genomic sequence.

5. The method according to Claim 1, wherein said method is a method of enhancing TERT expression.

6. The method according to Claim 5, wherein TERT expression is enhanced by inhibiting Myc Repeat repression of TERT expression.

7. The method according to Claim 6, wherein said inhibiting is by contacting said expression system with an agent that at least decreases the transcription repression activity of said Myc repeat region.

8. The method according to Claim 7, wherein said agent comprises a nucleic acid.

9. The method according to Claim 7, wherein said agent comprises a peptide or a protein.

10. The method according to Claim 7, wherein said agent is a small molecule.

11. A method for enhancing telomerase expression in a cell comprising a telomerase gene, said method comprising:

administering to said cell an effective amount of an agent that inhibits Myc Repeat TERT expression repression.

12. The method according to Claim 11, wherein said administering is *ex vivo*.

13. The method according to Claim 11, wherein said administering is *in vivo*.

14. The method according to Claim 11, wherein said method is a method for increasing the proliferative capacity of said cell.

15. The method according to Claim 11, wherein said method is a method for delaying senescence of said cell.

16. A method for enhancing telomerase expression in a mammal, said method comprising:

administering to said mammal an effective amount of an agent that inhibits Myc Repeat region repression of TERT expression.

17. The method according to Claim 16, wherein said agent is an agent that at least decreases the expression repression activity of said Myc Repeat region.

18. The method according to Claim 17, wherein said agent comprises a nucleic acid.

19. The method according to Claim 17, wherein said agent comprises a peptide or a protein.

20. The method according to Claim 17, wherein said agent is a small molecule.

21. The method according to Claim 16, wherein said method extends the lifespan of said mammal.

22. The method according to Claim 16, wherein said mammal is a human.

23. A method for decreasing telomerase expression in a cell comprising a telomerase gene, said method comprising:

administering to said cell an effective amount of an agent that enhances Myc Repeat TERT expression repression.

24. The method according to Claim 23, wherein said administering is *ex vivo*.

25. The method according to Claim 23, wherein said administering is *in vivo*.

26. A method for decreasing telomerase expression in a mammal, said method comprising:

administering to said mammal an effective amount of an agent that enhances Myc Repeat repression of TERT expression.

27. The method according to Claim 26, wherein said agent is an agent that at least enhances the transcription repression activity of said Myc repeat region.

28. The method according to Claim 27, wherein said agent comprises a nucleic acid.

29. The method according to Claim 27, wherein said agent comprises a peptide or a protein.

30. The method according to Claim 27, wherein said agent is a small molecule.

31. The method according to Claim 26, wherein said method is a method of treating a disease condition resulting from telomerase activity.

32. The method according to Claim 31, wherein said disease condition is characterized by abnormal cellular proliferation.

33. The method according to Claim 32, wherein said disease condition is cancer.

34. A nucleic acid present in other than its natural environment, wherein said nucleic acid has a nucleotide sequence that is the same as or substantially identical to the Myc repeat region and said nucleic acid does not include the full genomic TERT sequence.

35. The nucleic acid according to Claim 34, wherein said nucleic acid has a length ranging from about 1 to about 5000 bases.

36. The nucleic acid according to Claim 34, wherein said nucleic acid is isolated

37. The nucleic acid according to Claim 34, wherein said nucleic acid has a sequence that is substantially the same as or identical to a sequence found in a sequence selected from the group consisting of SEQ ID NOs:01 to 03.

38. An isolated nucleic acid or mimetic thereof that hybridizes under stringent conditions to the nucleic acid according to Claims 34 to 37 or its complementary sequence, wherein said isolated nucleic acid does not include the full TERT genomic sequence.

39. A construct comprising a nucleic acid according to Claims 34 to 38.

40. The construct according to Claim 39, wherein said construct comprises a TERT promoter.

41. The construct according to Claim 39, wherein said construct is an expression cassette.

42. A double stranded DNA decoy sequence comprising a Myc repeat region or portion thereof.

43. The decoy according to Claim 42, wherein said decoy comprises a sequence selected from the group consisting of SEQ ID NOs: 01 to 03 or portions thereof.

44. The decoy according to Claim 42, wherein said decoy ranges in length from about 10 to about 50 bases.

45. A method of treatment comprising administering to cells a decoy according to Claim 42.

46. A method of determining whether an agent that inhibits Myc repeat region repression of TERT transcription, said method comprising:

(a) contacting said agent with an expression system comprising a Myc repeat region and a coding sequence such that in the absence of said agent transcription of said coding sequence is repressed;

(b) determining whether transcription of said coding sequence is repressed in the presence of said agent; and

(c) identifying said agent as an agent inhibits Myc repeat repression of TERT transcription if transcription of said coding sequence is not repressed in the presence of said agent.

47. The method according to Claim 46, wherein said contacting step occurs in a cell-free environment.

48. The method according to Claim 46, wherein said contacting step occurs in a cell.

49. The method according to Claim 46, wherein said agent is a small molecule.

50. A mammalian cell comprising a telomerase gene modified by deletion of any of the nucleotides found in a Myc Repeat region.

51. The cell according to Claim 50, wherein said deletion is any of nucleotides found in a sequence selected from the group consisting of SEQ ID NOs: 01 to 03.

52. A method of producing a mammalian antibody, comprising the steps of:

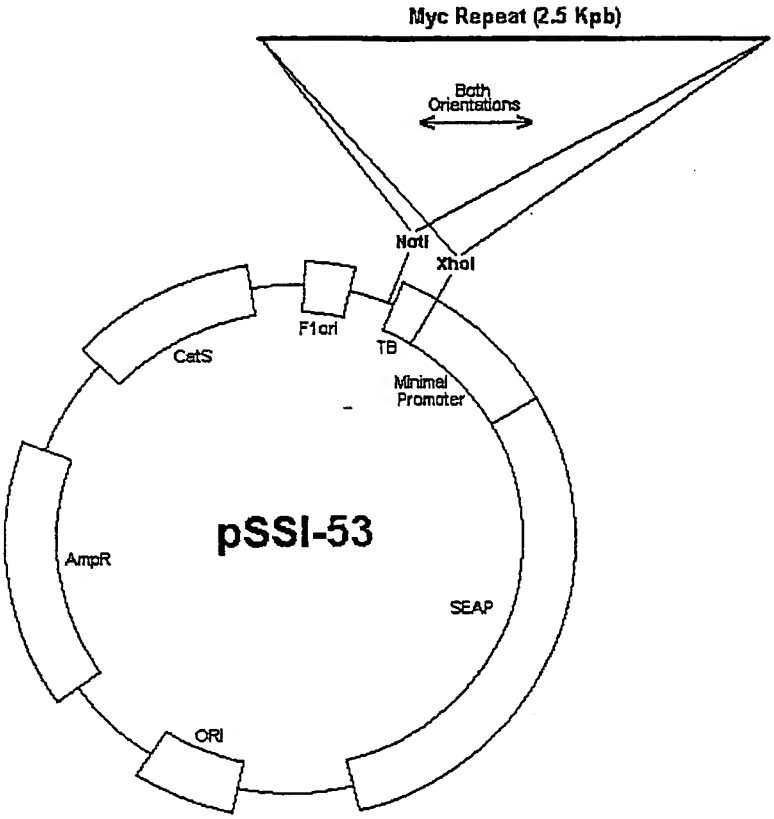
isolating a B cell from a mammal, which B cell or its progeny cell is characterized by producing an antibody of interest;

enhancing telomerase expression in said B cell by the method of Claim 11; and

growing the immortalized B cell and its progeny under conditions which allow the cells to produce the antibody of interest.

Figure 1

E-Box (Myc) Repeats



Plasmid	Insertion Site	Orientation
pSSI-53	No Insert	N/A
pSSI-107	XhoI	Sense
pSSI-108	XhoI	Anti-Sense
pSSI109	NotI	Sense
pSSI110	NotI	Anti-Sense

SEQUENCE LISTING

<110> Andrews, William H.
Foster, Christopher A.
Fraser, Stephanie
Mohammadpour, Hamid

<120> METHODS AND COMPOSITIONS FOR MODULATING TELOMERASE REVERSE TRANSCRIPTASE (TERT) EXPRESSION

<130> SIER-003WO

<150> 60/227,682
<151> 2000-08-24

<150> 60/227,681
<151> 2000-08-24

<160> 3

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 511
<212> DNA
<213> human

<400> 1
ccgggtgtcc ctgtcacgtg tagggtgagt gaggcgccgt ccccgggtgt ccctgtcacg 60
tgtagggtga gtgaggcgcc gtcccccggt gtccctgtca cgtgcagggt gagtgaggcg 120
ccgtccccgc gtgtccctgt cacgtgcagg gtgagtgagg tccgtccccg ggtgtccctg 180
tcacgtgtag ggtgagtgag gcgccgtccc cgggtgtccc tgtcacgtgt agggtgagtg 240
aggtgccgtc cccgggtgtc cctgtcacgt gcagggtgag tgaggccccg tccccgggtg 300
tccctgtcac attcagggtg agtgaggcgc ggtccccggg tgtccctgtc acgtgcaggg 360
tgagtgaggc gctgtccccg ggtgtccctg tcacgtgtag ggtgagtgag ttgcggcccc 420
cgggtgtccc tctcaggtgc agggtagtga ggcgctgtcc ctgggtgtcc ctgtctcgtg 480
tagggtgagt gaggctctgt ccccaggtgt c 511

<210> 2
<211> 586
<212> DNA
<213> human

<400> 2
cctggcttat gcagggagtg aggcgtggtc cccgggtgtc cctgtcacgt gcagggtgag 60
tgaggcgttg cccccaggtg tccctgtcac gtgtagggtg agtgaggcgc ggcccccggg 120
tgtccctgtc ccgtgcagcg tgattgaggt gtggcccccg ggtgtccctg tcacgtgtag 180
ggtgagtgag gcgccatccc cgggtgtccc tgtcacgtgt agggtgagtg aggcgtggtc 240
cccgggtgtc cctgtcccgt gcagggtgag tgaggcactg tccccgggtg tccctgtcac 300
gtgcagggtg agtgaggcgc ggtccccggg tgtccctctc aggtgtaggg tgagtgaggc 360
gcggccccag ggtgtccctg tcacgtgtag ggtgagtgag gcaccgtccc tgggtgtccc 420
tcccaggtat agggtgagtg aggcactgtc cccgggtgtc cctgtcacgt gcagggtgag 480
tgaggcgcgg cccccgggtg tccctctcag gtgcagggtg agtgaggcgc tgtccctggg 540
tgtccctgtc tcgtgtaggg tgagtgaggc tctgtcccca ggtgtc 586

<210> 3
<211> 1519
<212> DNA
<213> human

<400> 3
cccgggtgtc cctgtcacgt gtagggtgag tgaggcgcca tccccgggtg tccctgtcac 60

gtgtagggtg agtgaggcgt ggtccccggg tgtccctgtc ccgtgcaggg tgagtgaggc 120
actgtccccg ggtgtccctg tcacgtgcag ggtgagtgag gcgcggtccc cgggtgtccc 180
tgtcacgtgt agggtgagtg aggcactgtc cccgggtgtc cctctcaggt gtagggtgag 240
tgaggcgctg tccccgggtg tccctctcag gtgtagggtg agtgaggcgc ggccccaggg 300
tgtccctgtc acgtgtaggg tgagtgaggc accgtccctg ggtgtccctc ccaggtatag 360
ggtgagtgag gcactgtccc cgggtgtccc tgtcacgtgc agggtgagtg aggcgcggtc 420
cccaggtgtc cctgtcacgt gtagggtgag tgaggcactg tccccaggtg tccctgtcac 480
gtgcagggtg agtgaggcgc ggtccccagg tgtccctgtc acgtgcaggg tgagtgaggc 540
gccgtccccg ggtgtccctg tcacgtgcag ggtgagtgag gcacggcccc cgggtgtccc 600
tgtcacgtgc agggtgagtg aggcgcggcc cccgggtgtc cctgtcacgt gcagggtgag 660
tgaggcgccg tccccgggtg tctctgtcac gtgcagggtg agtgaggcgc cgtccccggg 720
tgtccctgtc acgtgcaggg tgagtgaggc acggcccccg ggtgtccctg tcacgtgcag 780
ggtgagtgag gcgcggcccc cgggtgtccc tgtcacgtgc agggtgagtg aggcgcggcc 840
cccgggtgtc cctgtcacgt gcagggtgag tgaggcgcgg tccccgggtg tccctgtcac 900
gtgcagggtg agtgaggcgc ggtccccggg tgtccctgtc acgtgcaggg tgagtgaggc 960
gcggtccccg ggtgtccctg tcacgtgcag ggtgagtgag gcacggtccc cgggtgtccc 1020
tgtcacgttc agggtgagtg aggcgcggtc cccgggtgtc cctgtcacgt gtagggtgaa 1080
tgaggcactg tccccgggtg tccctgtcac gtgcagggtg agtgaaggcg ccgtccccgg 1140
gtgtccctgt cacgtgcagg gtgattgacg cgaggccccc gggtgtccct gtcacgtgca 1200
gggtgagtga ggcgccgtcc ccgcgtgtcc ctgtcacgtg cagggtgagt gaggcgccgt 1260
ccccgggtgt ccctgtcacg tgtagggtga gtgaggcgcc gtccccgggt gtccctgtca 1320
cgtgtagggt gagtgaggcg ccgtccccgg gtgtccctgt cacgtgtagg gtgagtgagg 1380
cgccgtcccc gggtgtccct gtcacgtgta gggtgagtga ggcgccgtcc ccgggtgtcc 1440
ctgtcacgtg cagggtgagt gaggccccgt ccccgggtgt ccctgtcacg tgtagggtga 1500
gtgaggcact gtccccggg 1519

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/26039

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 3/00, 1/68; C12P 21/04; C12N 15/00, 5/00; C07H 21/04
US CL : 435/3, 6, 7.21, 320.1, 325; 514/44; 424/93.1; 536/ 24.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/3, 6, 7.21, 320.1, 325; 514/44; 424/93.1; 536/ 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, CAPLUS, USPATFUL, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HORIKAWA, I. et al. Cloning and characterization of the promoter region of Human Telomerase. Cancer Research. 15 February 1999, Vol. 59, pages 826-830, see entire document.	1-22
A	WICK, M. et al. Genomic organization and promoter characterization of the gene encoding the human telomerase reverse transcriptase (hTERT). Gene. 1999, Vol. 232, pages 97-106, see entire document.	1-22
A	CROWE, D.L. et al. E2F-1 represses transcription of the human telomerase reverse transcriptase gene. Nucleic Acid Research. 2001, Vol. 29, No. 13, pages 2789-2794, see the entire document.	1-22

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
06 November 2001 (06.11.2001)

Date of mailing of the international search report
31 DEC 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703)305-3230

Authorized officer
Ram R. Shukla

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/26039

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-22

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/26039

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-22, drawn to a method of enhancing expression of TERT in an expression system comprising a Myc Repeat region in vivo and ex vivo.

Group II, claim(s) 23-33, drawn to a method of decreasing telomerase expression in a cell ex vivo and in vivo.

Group III, claim(s) 34-41, drawn to a nucleic acid comprising a nucleotide sequence that is same or identical to Myc repeat region.

Group IV, claim(s) 42-45, drawn to a double stranded DNA decoy sequence comprising a Myc repeat region.

Group V, claim(s) 46-49, drawn to a method of screening for agents that inhibit Myc repeat repression of TERT transcription.

Group VI, claim(s) 50 and 51, drawn to a mammalian cell comprising a telomerase gene that comprises a deletion of Myc repeat region.

Group VII, claim(s) 52, drawn to a method of producing a mammalian antibody.

The inventions listed as Groups I-VII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The invention of group I requires an agent that enhances TERT expression whereas the method of group II requires an agent that inhibits TERT expression. Therefore the inventions of groups I and II lack the same special technical feature.

The invention of group III is a nucleic acid sequence that is identical to Myc repeat region and would include nucleic acid sequences that would not function as Myc repeat region and therefore, it lacks the same technical feature as groups I and II.

The invention of group IV is a double stranded decoy which is not required for the methods of groups I-II and therefore, lacks the same technical feature as that of groups I-II. Furthermore, its structure is distinct from that of the nucleic acid of group III.

The invention of group V lacks the same special technical feature because the steps of groups I-III can not be used to practice this method and this method can be used for making the compositions of groups III and IV.

The invention of group VI is drawn to a mammalian cell that can not be made by the methods of the groups I-II and V and its structure is distinct from that of the the compositions of groups III and IV.

The invention of group VII is for producing an antibody and its steps can be used for practicing the methods of groups I and II or vice versa. Furthermore, the compositions of groups III, IV, and V can not be used in this method.